

SEM Studies on Vessels in Ferns. 4. *Astrolepis*

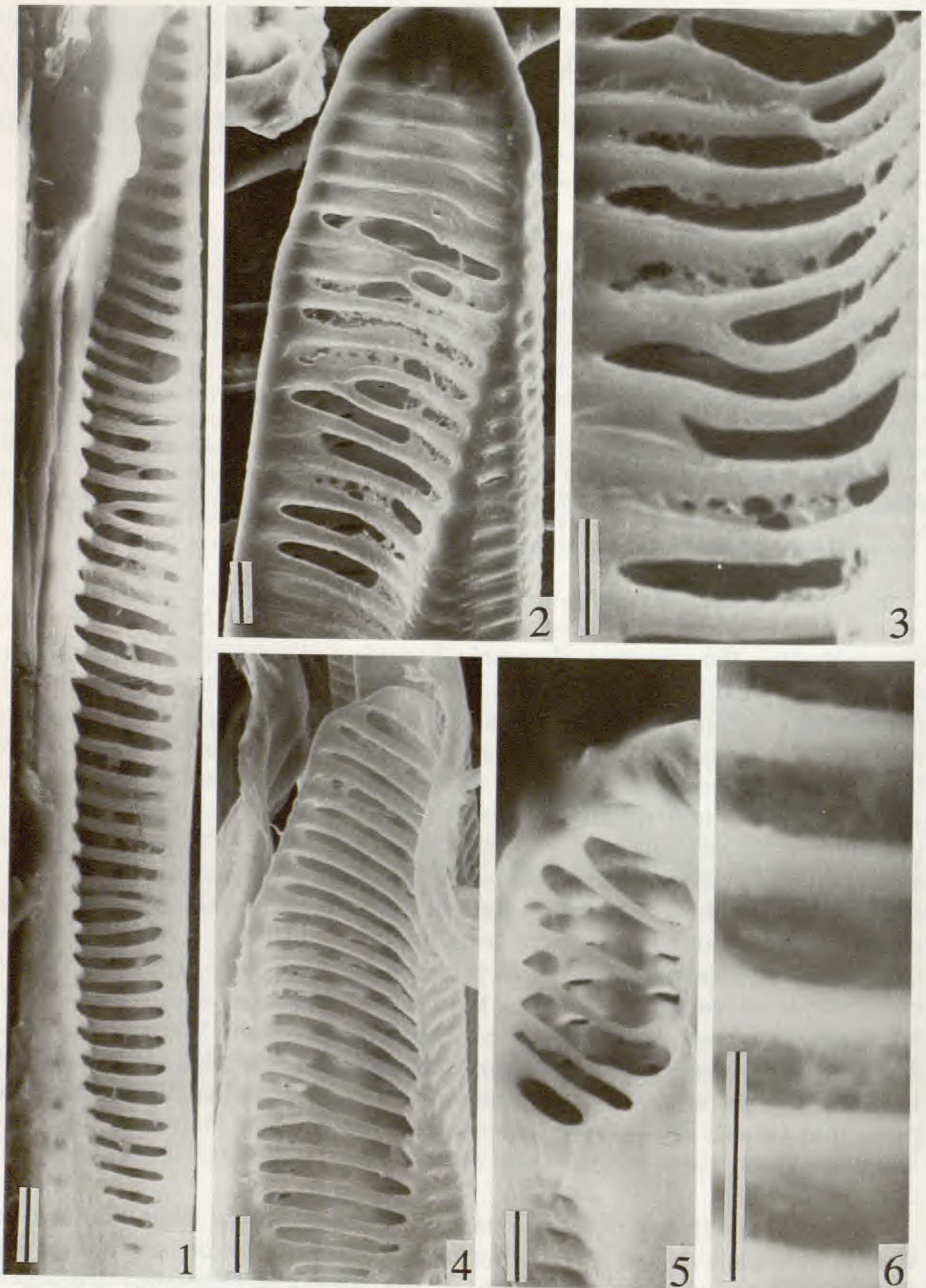
SHERWIN CARLQUIST and EDWARD L. SCHNEIDER

Santa Barbara Botanic Garden, 1212 Mission Canyon Road, Santa Barbara, CA 93105

ABSTRACT.—By means of scanning electron microscopy, the nature of vessels is described for roots and rhizomes of *Astrolepis sinuata* (Lag. ex Swartz) D.M. Benham & Windham [= *Notholaena sinuata* (Lag. ex Swartz) Kaulf.], commonly placed in Pteridaceae. In both roots and rhizomes, end walls are variously distinct from lateral walls; a few perforation plates are quite elongate with numerous bars, but most are of medium or short length with fewer bars. Pit membrane remnants are generally sparse in the perforation plates. Lateral wall perforation plates are present; some may represent a multiplicity of facets at the tip of a tracheary element. Lateral walls may have intact pit membranes or perforations. No tracheids were identified; apparently all tracheary elements are vessel elements. The abundance of relatively short end walls with few bars on perforation plates may be correlated with rapid rates of water conduction during the short growing season of this fern, which occupies outcrops in arid parts of the southwestern United States and Mexico.

Scanning electron microscopy (SEM) offers a highly effective method of demonstrating the presence of vessels, because with this method pores large enough to constitute perforations can be seen. We have been attempting to describe the nature and extent of vessels in ferns by means of SEM (Carlquist et al., 1997; Carlquist and Schneider, in press; Schneider and Carlquist, 1997). Our studies have taken as a point of departure the studies of White (1962, 1963), in which end walls appreciably different from lateral walls were recorded in tracheary elements of particular ferns. White (1962) suspected that some of these tracheary elements might be vessels. *Astrolepis sinuata* was a species for which White (1962) figured tracheary elements that have "end plates" different in their patterns from lateral wall pitting. Light microscopy cannot reveal presence of pores in pit membranes of ferns, and thus White's conclusions on vessel presence on the basis of light microscopy were speculative except where clear presence of vessels is concerned. White (1961, 1962) accepted the presence of vessels in *Pteridium* and *Marsilea*. Because of the inability of light microscopy to resolve perforations, earlier literature has reports that vessels are present in some ferns other than *Pteridium* (Gwynne-Vaughan, 1908); these reports were contradicted by Bancroft (1911) and Duerden (1940).

In extending White's observations, we can confirm that there are clear instances of vessels in which scalariform perforation plates lacking any vestiges of pit membranes are present. Some of the vessels of *Pteridium* conform to this pattern (Carlquist & Schneider, in press), although some vessels in that species retain pit membranes that are porose; these porosities qualify such tracheary elements as vessels. In some ferns, only porose pit membranes occur on end walls; this is true of *Woodsia obtusa* (Sprengel) Torrey (Carlquist et al., 1997). Perforations of this sort on lateral walls of vessels were reported in



FIGS.1-6. SEM photographs of tracheary elements from macerations of roots of *Astrolepis sinuata*, showing perforation plates or portions of perforation plates; concave conformations in some of these plates are artifacts. 1) Long perforation plate with few pit membrane remnants between bars. 2) End wall of medium length, with some intact pit membranes and with perforations that have variously porose pit membranes; imperforate lateral wall pitting, lower right. 3) Portion of a perforation plate, showing some pit membrane remnants in which small pores are evident. 4) End

Pteridium (Carlquist and Schneider, in press) and in *Phlebodium* and *Polystichum* (Schneider and Carlquist, 1997). In the latter two genera, perforations free from pit membranes as well as perforations that retain pit membranes were observed (Schneider and Carlquist, 1997). Thus, a range of expressions in vessel presence is now known to occur in ferns.

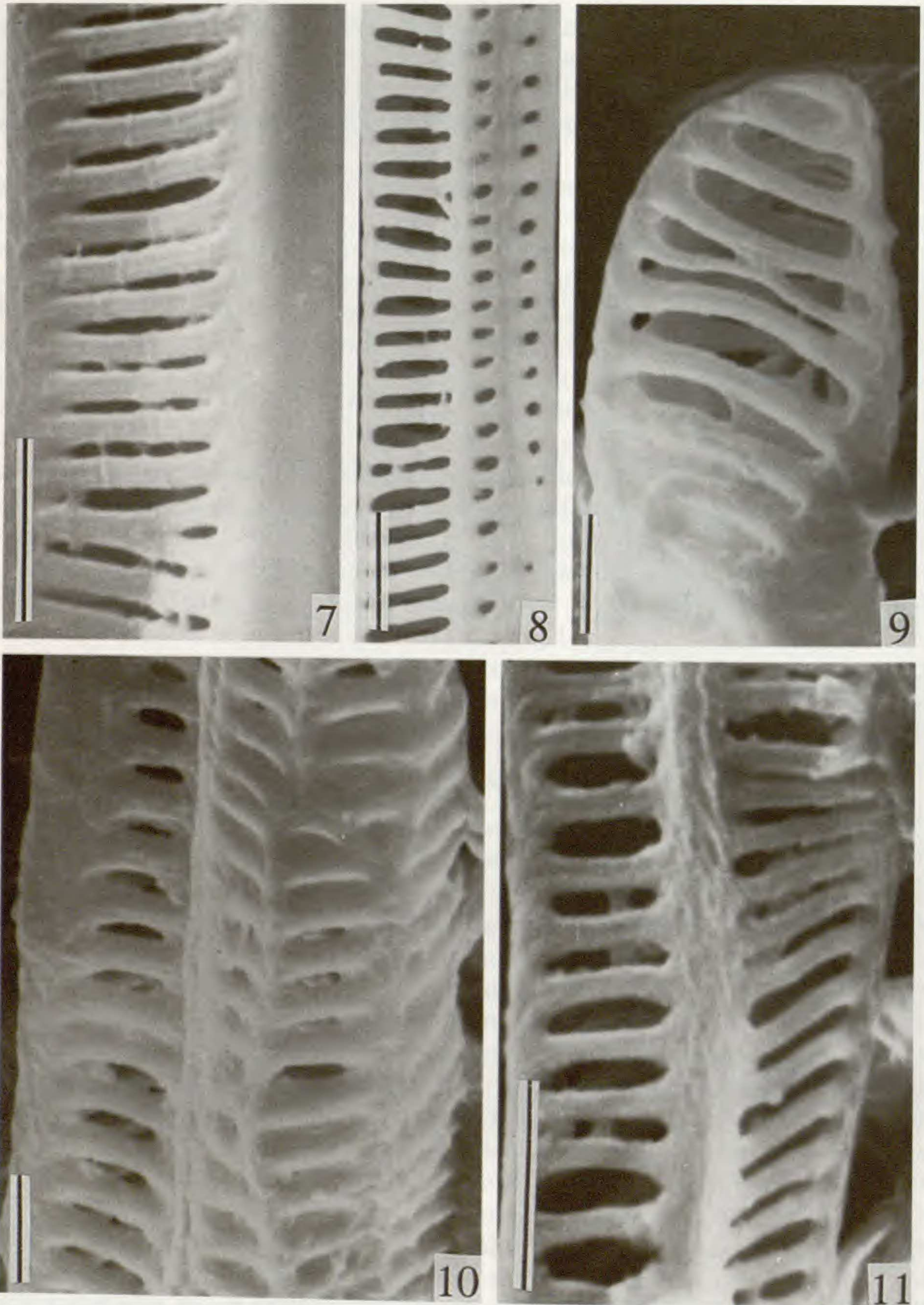
The diverse patterns of vessel presence and morphology discovered thus far by means of SEM suggest that vessels are much more widespread in ferns than hitherto suspected. In turn, phylogenetic and physiological implications are raised. Are vessels polyphyletic in ferns, and if so, have they originated many or few times? Do the presence of vessels in particular ferns and the morphology of those vessels relate to the ecological regimes to which those species are adapted, respectively? What is the physiological significance of porose pit membranes, e.g., can air embolisms traverse such pores? Are porose membranes better than imperforate membranes at conduction of water in xylem? Why do some ferns have porosities in pit membranes of lateral walls, and what adaptive significance do lateral-wall perforation plates have? Although we cannot answer all of these questions on the basis of our SEM studies, we believe we can provide a data base that can contribute to an improved understanding of phyletic and physiology of fern xylem. *Astrolepis sinuata* is a fern of particular significance with regard to ecological significance of vessels, because it occurs in dry areas (usually in rock outcrops) from western Texas to southeastern California and to South America (Lellinger, 1985).

MATERIALS AND METHODS

Our material of *Astrolepis sinuata* was cultivated in the Botanic Garden of the University of California at Berkeley. This plant was derived from a plant cultivated at the New York Botanic Garden, which was grown from a plant collected in the wild by J. Beitel and J. Mickel (s.n.), 23 Jan 1989, on a hill called Las Cruces at Tlacolulu, at the intersection of Route 190 with Route to Macuilxochitl, east of Oaxaca; on steep rocky hill, 1600 m, det. by A. Smith. Living material was sent to us; we preserved portions in 50% aqueous ethanol. Macerations of roots and stems were prepared with Jeffrey's Fluid, stored in 50% aqueous ethanol, spread over the surface of aluminum stubs, sputter-coated, and examined with a Bausch and Lomb Nanolab SEM. Paraffin sections were prepared according to the usual techniques, mounted on glass slides, and stained with Northen's modification of Foster's tannic acid-ferric chloride method (Johansen, 1940). These sections were useful in seeing the vascular structure of the root and stem with light microscopy and thereby offering data that could be compared to findings made with SEM. Our earlier work with *Pteridium* (Carlquist and Schneider, in press), in which we examined both

←

wall of medium length, in which very few pit membrane remnants are present; imperforate lateral wall pitting at right. 5) Short end wall with few bars on perforation plate and no pit membrane remnants. 6) Weblike pit membranes in perforations of an end wall. Scale bars=10 μ m.



FIGS. 7-11. End walls (Figs. 7-9) and lateral walls (Figs. 10-11) from tracheary elements of rhizome of *Astrolepis sinuata*. 7) Portion of perforation plate with pit membrane remnants in most of the perforations. 8) End wall perforation plate with few remnants in perforations, left, and two narrower perforation plates, right. 9) Short end wall in which the perforation plate has few, wide bars. 10) Lateral walls of two adjacent tracheary elements; most of the lateral wall faces of the element at left lack pores, but the lateral wall of the element at right is highly porous. 11) Two

sectioned and macerated xylem with SEM, convinced us that the data obtained with the two methods are identical. Because fern tracheary elements have hard secondary walls and break excessively when sectioned longitudinally, we have elected to use macerations as the source of observations in the present paper.

We judge all of the tracheary elements figured to be quite mature, because stem portions selected for maceration were well distal to the apical meristem, and root portions selected for maceration were adjacent to the rhizome. Thus, we believe that tracheary elements with porose pit membrane remnants in perforations are comparable in degree of maturity to tracheary elements with perforations free from pit membrane remnants.

RESULTS

Tracheary elements of roots (Figs. 1–6) are likely all vessels, because we could not find any tracheary elements clearly lacking porosities in end walls. The perforation plates of vessels, however, range widely in morphology. Some plates are very long with numerous bars and only a few remnants of primary wall material (Fig. 1). Some end walls are shorter (Fig. 2), and feature pits without pores (Fig. 2, top and bottom) as well as perforations in which lysis has produced small to large pores (Fig. 2, center). Perforations often feature series of circular to oval pores, with various degrees of coalescence to form large holes (Fig. 3). One can find all sizes of pores, ranging from some nearly as large as perforations to some that barely be resolved with our equipment (Fig. 3). In some perforation plates of medium length, remnants of pit membranes are entirely absent (Fig. 4). Some perforation plates have very few and narrow bars (Fig. 5). All of the perforation plates in Figures 1–5 are markedly wider than the relatively narrow cell faces presented by lateral wall pitting (Fig. 2, right; Fig. 4, right). In some end walls, however, delicate weblike pit membrane remnants were noted (Fig. 6). Although pores in lateral wall pits of tracheary elements of roots are not figured, we noted a few instances of this phenomenon.

In tracheary elements of rhizomes (Figs. 7–11), there is a range in perforation plate morphology similar to that seen in roots. The end wall shown in Fig. 7 shows a perforation plate similar to lateral wall pitting: the bars between the perforations are wide, but most of the perforations are relatively free from pit membrane remnants. At right in Fig. 7 is a smooth, unpitted portion of the vessel wall. Although macerations are not suitable for determination of contacts between particular cell types, we believe that this smooth wall represents a vessel-parenchyma interface because parenchyma has inconspicuous primary pit fields that do not have a scalariform disposition. Scalariform lateral wall pitting is therefore a reliable indicator that a tracheary element cell face was adjacent to a similar face of another tracheary element. Parenchyma cells

←

lateral walls of a tracheary element, showing prominent perforations, relatively few pit membrane remnants. Scale bars=10 μ m.

were observed in our macerations, but have not been figured here. The vessel element in Fig. 8 shows similarity between an end wall with a wider perforation plate at left and two narrower perforation plates at right. One could say that in this vessel element, there are several perforation plates that contact perforation plates of other vessel elements superposed or even lateral to the one shown. The distinction between a perforation plate and lateral wall pitting disappears in such a vessel element. Also present in the rhizome are some vessel elements with clear differentiation between end walls and lateral walls, as in the vessel element shown in Fig. 9 (lateral walls not shown in this figure, but similar to lateral walls of Fig. 10). The perforation plate consists of few and wide bars.

Lateral walls of rhizome tracheary elements are shown in Figs. 10, 11. In Fig. 10, there are two tracheary elements. In the one at left, relatively few pores penetrate the pit membranes. More numerous and wider openings in the pit membranes occur in the lateral wall shown for the tracheary element at right. In Fig. 11, absence of pit membrane remnants is notable; one must call these surfaces perforation plates on this account. Thus, in *Astrolepis sinuata* there are all degrees of cell wall openings from imperforate lateral wall pits to perforation plates.

DISCUSSION AND CONCLUSIONS

In both roots and rhizomes, perforation plates in *Astrolepis sinuata* range from little differentiated, with abundance of porose pit membrane remnants, to scalariform perforation plates with few bars and no pit membrane remnants. There are also lateral perforation plates, although not all lateral walls facing other tracheary elements have porose pit membranes: some of these lateral walls retain intact pit membranes. All tracheary elements appear to have perforation plates, and no tracheids were identified with certainty. All of these features can be found in *Pteridium* (Carlquist and Schneider, in press) as well as *Phlebodium* and *Polystichum* (Schneider and Carlquist, 1997). Tracheary elements of *Woodsia obtusa* (Carlquist et al., 1997) have no lateral perforation plates, and the end walls are relatively little differentiated as perforation plates; pit membrane remnants are present, and are moderately porose. However, these features should not be considered as differences between the genus *Woodsia* and the other genera. Other species of *Woodsia*, such as *W. ilvensis* (L.) R. Brown, very likely when studied with SEM will prove to have perforation plates as specialized as those of *Pteridium*, if not more so, judging from the light microscope studies of White (1962). Parenchyma-vessel interfaces of *Astrolepis sinuata* are notable for absence of pitting; examination of transections of the meristemes shows that parenchyma is relatively abundant within the xylem of this species.

The degrees of specialization of perforation plates in the ferns we have studied to date are not related to taxonomic position. The development of vessels in ferns, like that in monocotyledons, seems likely to relate to ecology. As yet, the number of ferns we have studied is insufficient to develop an outline of

the relationship between types of tracheary elements and ecology. We believe that such a relationship, as with monocotyledons (Carlquist, 1975) is likely. As patterns of relationship between xylem structure and ecology become clearer, implications for physiology of fern xylem will emerge. Thus, our survey of fern xylem can offer the basis for valuable advances in understanding of fern physiology.

We have begun this series of papers with studies of ferns in which vessel presence could likely be demonstrated when studied with SEM, rather than ferns in which xylem might prove to consist of tracheids only. Certainly *A. sinuata* qualifies as a xeric fern. Therefore, vessels with simplified (fewer bars, absence of pit membranes), as found in this species, seem to correlate with conduction of larger water volumes during the short season during which soil moisture is abundant.

Tracheary elements with a greater degree of differentiation of end walls from lateral walls might be expected in roots, rather than rhizomes of ferns, judging from the data of White (1962; see also Carlquist et al., 1997). Although observation of SEM preparations does not permit development of quantitative data on this point, we note that in all of the ferns we have studied to date, vessel elements in both roots and rhizomes have a range of morphology from tracheid-like to moderately specialized. Future studies are needed to establish whether the degrees of specialization of perforation plates in roots as compared to rhizomes are about the same within a given fern species, or whether, as in Cheadle's (1942) study of monocotyledons, there is greater specialization in perforation plates of roots as compared to those of stems.

ACKNOWLEDGMENT

We are grateful to Holley Forbes, Assistant Curator of the Botanic Garden of the University of California at Berkeley, for sending us the plants used in this study.

LITERATURE CITED

- BANCROFT, W. 1911. On the xylem elements of the Pteridophyta. *Ann. Bot. (Oxford)* 25:745-759.
- CARLQUIST, S. 1975. *Ecological strategies of xylem evolution*. University of California Press, Berkeley and Los Angeles.
- CARLQUIST, S., and E. L. SCHNEIDER. In press. SEM studies on vessels in ferns. 2. *Pteridium*. *Amer. J. Bot.*
- CARLQUIST, S., E. L. SCHNEIDER, and G. YATSKIEVYCH. 1997. SEM studies on vessels in ferns. 1. *Woodsia obtusa*. *Amer. Fern J.* 87:1-8.
- CHEADLE, V. I. 1942. The occurrence and types of vessels in the various organs of the plant in the Monocotyledoneae. *Amer. J. Bot.* 29:441-450.
- DUERDEN, H. 1940. On the xylem elements of certain ferns. *Ann. Bot. (Oxford)* 4:523-531.
- GWYNNE-VAUGHAN, D. T. 1908. On the real nature of the tracheae in the ferns. *Ann. Bot. (Oxford)* 22:517-523.
- JOHANSEN, D. A. 1940. *Plant microtechnique*. McGraw Hill, New York.
- LELLINGER, D. B. 1985. *A field manual of the ferns & fern-allies of the United States & Canada*. Smithsonian Institution Press, Washington, DC.
- SCHNEIDER, E. L., and S. CARLQUIST. 1997. SEM studies on vessels in ferns. 3. *Phlebodium* and *Polystichum*. *Int. J. Pl. Sci.* 158:341-347.

- WHITE, R. A. 1961. Vessels in roots of *Marsilea*. *Science* 133:1073-1074.
- . 1962. A comparative study of the tracheary elements of the ferns. Ph.D. dissertation, University of Michigan, Ann Arbor.
- . 1963. Tracheary elements of the ferns. II. Morphology of tracheary elements; conclusions. *Amer. J. Bot.* 50:514-522.